STARCH SYNTHESIS IN THE VARIEGATED LEAVES OF PELARGONIUM.[1]

A. G. CHAPMAN AND W. H. CAMP.

INTRODUCTION.

Under natural conditions, the non-green portions of most variegated leaves do not synthesize starch. It has, however, been known for some time that the non-green portions of many leaves could synthesize starch if artificially supplied with glucose.

In the green leaf, the more important underlying conditions known to affect starch synthesis are: the concentration of soluble carbohydrates; the type and distribution of plastids; the activity of certain enzymes, particularly the carbohydrases; and the hydrogen-ion concentration of the cell contents.

Böhm (1883) was probably among the first to show that green leaves would synthesize starch in the chloroplasts if floated on a sugar solution in the dark. Spoehr (1926) mentions similar investigations by Meyer (1886), and others. Saposchnikoff (1889) and Borkorny (1897) also showed that starch synthesis would occur in starch-free green leaves of many species if floated on solutions of soluble carbohydrates in the dark. Miller (1931) reports that Böhm (1883), Schimper (1885), and Meyer (1886) found that starch formation occurred in chloroplasts and leucoplasts only when the solution of sugar had reached a certain concentration. Winkler (1898) concluded that when the concentration of glucose or sucrose is sufficiently high, all chloroplasts and leucoplasts, with but few exceptions, form starch. He found that the lowest concentration of sucrose which induced starch formation in most cases was 0.02 percent, the optimum 10 percent; above this the starch decreased until in a 30 percent solution of sucrose no starch was produced. Spoehr (1926) concluded that the concentration of glucose necessary for starch formation varies widely for different plants. Lundegardh (1914) claimed that the transformation of sugar to starch in cells is very complicated, and that the process depends not only upon the

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concentration of sugar in the cytoplasm, but also upon the amount of an enzyme, the concentration of which is controlled by unknown factors. Spoehr (1926), pointed out that various workers have found that plants form starch in the dark when floated on solutions of not only glucose, fructose, galactose, mannose, sucrose, and maltose, but also of such alcohols as mannitol, ducitol, erythritol, and glycerol. According to Winkler (1898), formaldehyde and an extract from natural humus may also be used.

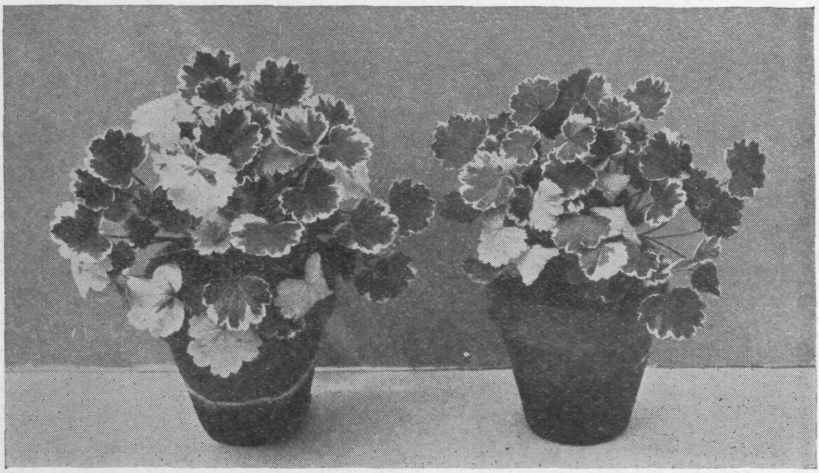


FIG. 1. *Pelargonium* plants from the clon culture used in the experiments.

A number of investigators have studied the ability of various plants to synthesize starch in the non-green portions of their leaves, and also certain of the factors which normally inhibit its formation. Winkler (1898), after unsuccessful attempts to induce starch synthesis in the white portions of the leaves of *Pandanus Veitchii*, found there were no plastids present. Hein (1926) in a review of the subject quotes Woods (1899) as believing that in *Pelargonium* and in many other cases of variegated leaves, the chlorophyll is destroyed by oxydizing enzymes, the oxidases and peroxidases, which are normally present in all green plants and under certain conditions may be produced in abnormally large quantities. He also states that Baur (1904) found that the plastids in the white cells of *Pelargonium* uniformly contain little or no chlorophyll and are smaller than the plastids in the green cells, and that

Küster (1919) concluded that the plastids in the non-green areas of *Pelargonium* are incompletely developed or have become bleached and degenerate, possibly through agencies inside the cells.

Maige (1924), studying the effect of temperature on starch formation in various leaves, found within certain limits, a correlation between the two. Henrici (1921) showed that the minimum temperature and light intensity necessary for photosynthesis are lower than those required for starch formation. Gillis (1923) thought that the formation of starch from sugar in variegated leaves depends to a certain extent on light.

The work done by the present writers was undertaken as a further study of the factors which inhibit starch formation in non-green leaf tissue under normal greenhouse conditions, and of the factors which affect the rate of starch formation when carbohydrates are supplied.

In the preliminary work, various species of variegated-leaved plants were used. The present report, however, is found in studies of the variegated geranium (*Pelargonium hortorum* var. *Mme. Salleroi*).

The writers wish to acknowledge the help of Dr. H. F. Thut of the Alabama Polytechnic Institute, who assisted in the preliminary part of the investigation.

SUGAR CONCENTRATION OF SUBSTRATE.

In this portion of the investigation, the material consisted of carefully selected *Pelargonium* leaves obtained from plants of a clon culture. (Fig. 1).

METHODS.—Mature, but active leaves from representative plants were removed with a sharp razor. The cut ends of the petioles were immediately immersed in freshly prepared glucose solutions of 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 molecular concentration.[2] A sufficient number of albino as well as variegated leaves were available for the study. Two series were made, one being placed in the dark, the other in the light, the temperature and atmospheric humidity being nearly equal in both situations.

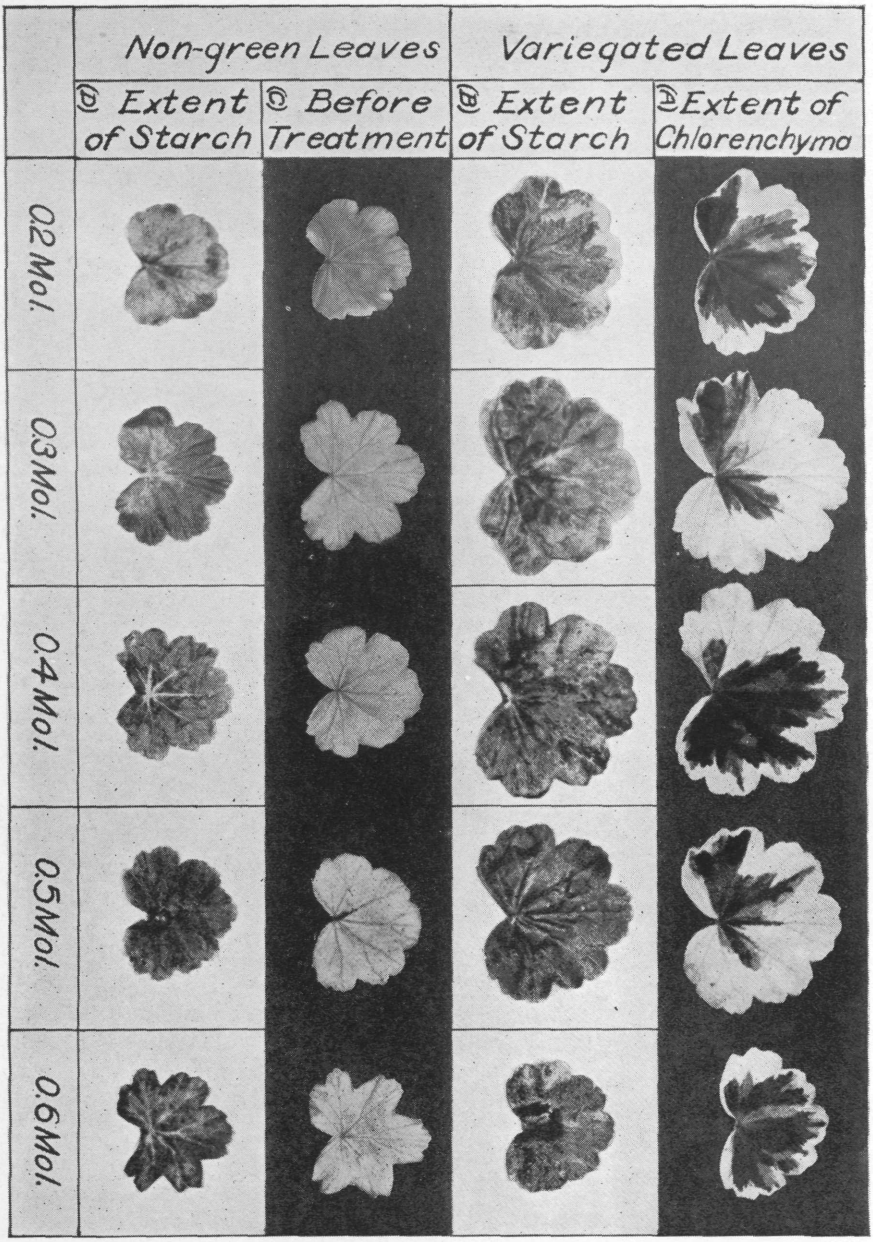
After fifteen hours, the chlorophyll was removed from the leaves by means of ethyl alcohol and starch tests made by the usual iodine method.

[2]All glucose solutions were weight molecular from C. P. material.

RESULTS.—The following results were obtained in both the variegated and albino leaves. A trace of starch was present in the leaves which were in the 0.2 molecular solutions. In the higher concentrations, the amount of starch in the leaves increased proportionately with the concentration of the glucose up to the 0.5 molecular solution. In the 0.6 molecular solution a slight decrease was noted, and only a trace of starch appeared in the 0.7 molecular solution. Slight effects of plasmyolysis were observed in a few of the leaves in the 0.6 molecular solutions; and wherever this condition was found, a noticeable decrease in the starch was observed. The small quantity of starch in the 0.7 molecular concentration probably was due to the conditions accompanying plasmolysis which was most severe in the albino portions. Non-green portions of the leaves in 0.3, 0.4, 0.5 and 0.6 molecular concentrations, except where noted for the 0.6 molecular solution, became dark violet in color on the application of the iodine solution. No great difference could be observed between the green and non-green portions of the leaves after testing; the transition line between the two portions disappeared because of the uniform distribution of the starch. (Fig. 2.)

No noticeable difference occurred in the amount of starch synthesized by leaves in the dark or in the light.

DISCUSSION.—While Winkler (1898) and others selected glucose and sucrose as the two most desirable materials for synthesizing starch in leaf tissues, they employed almost exclusively a 10 percent solution of sucrose. Winkler does not state the time required for starch formation in starch-free *Pelargonium* leaves; but for other species, the time varied from one day to three weeks. To keep the floating leaf parts as free as possible from bacteria and fungous growth, he occasionally transferred the material to fresh sugar solutions which were treated with a sufficient amount of phosphoric acid for sterilization. In many cases, there was a continuous increase of starch in the leaf tissue over a period of two to three days. This delay and comparatively long period of gradual increase might have been closely correlated with the rate of hydrolysis of sucrose in the acid solution. It has been well established by Weevers (1924) and others that starch is not synthesized directly from sucrose. It is therefore the opinion of the writers that in the earlier work, where sucrose was used as a substrate,



Series of leaves of *Pelargonium* showing the amount of starch synthesized in 15 hours when the cut petioles are immersed in varying molecular concentrations of glucose. Rows A and B are from variegated leaves before and after treatment. Rows C and D are from albino leaves.

that it was not sucrose that was used by the plant in starch synthesis but its hydrolytic products.[3]

It was known long before the investigations of Winkler (1898) that light is not necessary for starch synthesis. Yet Winkler thought that there might be an indirect influence of light upon the starch building process, such as, "a promoting of chloroplasts" and "a retarding of leucoplasts." Miller also mentions that "The formation of starch might be influenced by light in an entirely different manner from that influencing the process of photosynthesis."

The writers' results obtained from *Pelargonium* leaves in both daylight and complete darkness show no observable difference in the quantity of starch synthesized in the non-green portion. At least for this species, any indirect beneficial or deleterious effects of light, due either to intensity or quality, are probably insignificant or lie outside the range of intensity and quality of light experienced under greenhouse conditions.

SUGAR DETERMINATIONS.

As the preliminary work showed that starch synthesis would occur in the non-green portions of *Pelargonium* leaves when glucose was supplied, the following study was made to ascertain the relative amounts of sugar normally present in the several parts of the leaf.

METHODS.—Total sugar determinations were made, according to the Shaffer-Hartman method, on duplicate samples of both green and non-green portions of the same leaves. The samples were collected shortly before noon on a clear day and transferred at once to 80 percent ethyl alcohol which was soon brought to the boiling point to prevent further enzyme action. Five-tenths of a gram of calcium carbonate was also added to neutralize any acids present. The amounts of total sugars were calculated from the standard Munson-Walker tables.

As it has been established that starch may be synthesized from several of the soluble carbohydrates or their hydrolitic products, no attempt was made to differentiate between the reducing and non-reducing sugars, the results being expressed as total sugars.

RESULTS.—Expressed in the percentage of the fresh weight,

[3]It may be noted that the writers were able to obtain starch synthesis when fructose was used as a substrate.

samples from the green portion contained 0.36% total sugars, while the albino portions contained only 0.18% total sugars.

DISCUSSION.—Analyses by the writers have shown that the total sugar content of the green tissue at mid-day was approximately twice that of the non-green part. It is generally known that there is a point of sugar-starch equilibrium above which starch formation exceeds starch hydrolysis, and that this point of equilibrium may shift with certain internal factors. The fact that starch synthesis is induced by artificially supplying glucose, leads the writers to believe that the sugar concentration and its relation to the sugar-starch equilibrium is an important factor in the lack of starch formation in the non-green leaf portions of *Pelargonium*.

FLUCTUATION IN HYDROGEN-ION CONCENTRATION.

The well-known effect of hydrogen-ion concentration on many physiological reactions prompted the writers to make a study of this factor in relation to the problem of starch synthesis. Determinations of the hydrogen-ion concentrations were made to show the changes in pH over a period of twenty-four hours.

METHODS.—In the study of the pH fluctuation over a twenty-four hour period, pots in which the plants were growing were sunk two-thirds of their depth in sand. The plants and sand were watered to insure a sufficient water supply during the course of the experiment.

Starting at 12 noon on May 26, 1931 samples of the green and non-green portions of the leaves were taken every three hours until noon the next day. Thirty-six plants, derived from a clon culture, were used. During the entire period of the experiment the sky was clear. The samples were obtained by separating the green and non-green portions of representative leaves with scissors until enough material was obtained to make a determination. Twenty-five leaves were used for each determination.

The separate samples from the two parts of the leaves were placed in one-inch, rubber-stoppered test tubes and immersed in a bath at —20 C., where they were quickly frozen. After ten minutes the samples were thawed and the juice expressed by means of a power press at a standard pressure of 2500 pounds per square inch. It was only by this method that a sufficient quantity of extract could be obtained to make a

determination. The quinhydrone potentiometer was used to find the hydrogen-ion concentration of the expressed juice. Since none of the pH values found closely approached the pH of 7.0, there was probably no decomposition of the quinhydrone sufficient to produce anomalous results.

Determinations of fluctuations in pH over a 24-hour period in the variegated coleus (*Coleus Blumeii*) were made at the same

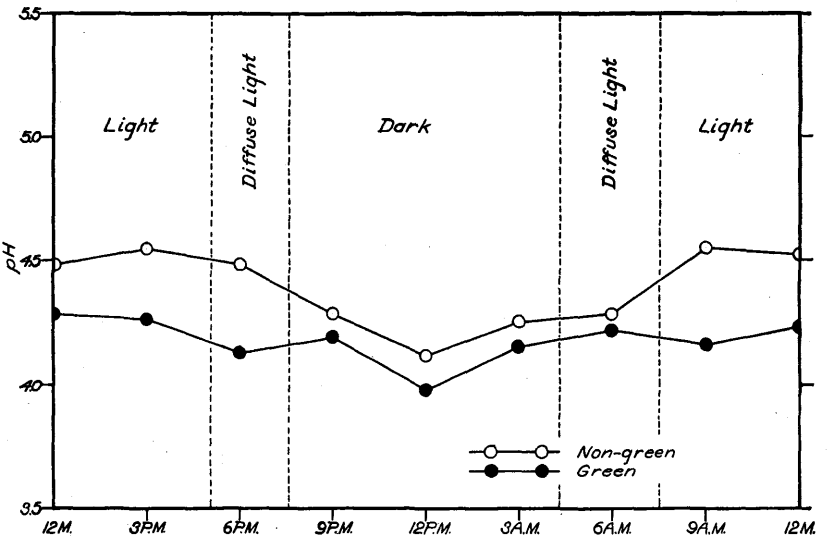


FIG. 3. Comparison of the fluctuations of the pH values in the green and non-green portions of the variegated leaves of *Pelargonium* over a 24-hour period.

time. Determinations of the relative pH of the margins and central portions of the non-variegated leaves of cabbage (*Brassica* sp.) were also made.

RESULTS.—Figure 3 shows the fluctuation in pH in the green and non-green portions of the leaves during a twenty-four hour period. It is evident that although fluctuations occur, the non-green portion of the leaves was consistently higher in pH than the green portion.

In the study of pH fluctuation in the variegated coleus it was found that the non-green inner portion of the leaf varied between 5.10 and 5.95, while in the outer green portion the pH varied between 5.85 and 6.15.

The cabbage without variegated leaves, showed a pH

value of 5.85 at the margin of the leaf and a pH value of 5.72 in the central portion.

DISCUSSION.—It is clear from the above results that the outer (non-green) portions of *Pelargonium* leaves are consistently higher in pH than the inner (green) portions. Studies of the variegated-leaved coleus, in which the position of the green and non-green areas is the reverse of those of *Pelargonium*, as well as of the entirely green leaves of cabbage, gave similar results. Freeland (1931) also found that the leaf margins of *Bryophyllum calycinum* and *B. crenatum* had a higher pH value than the central portion. These various results led the writers to believe that in the species examined the decreasing gradient of pH from the leaf margin to the center is not so much correlated with the green or non-green tissues as with their position in the leaf.

HYDROGEN-ION CONCENTRATION OF THE SUBSTRATE.

In the previous experiments on the fluctuation in H-ion concentration, the writers found a difference between the green and non-green portions of *Pelargonium* leaves. It was thought that this difference might influence the point of sugar-starch equilibrium. Therefore, a study was made to determine, if possible, whether a difference in the H-ion concentration of the substrate on which the leaves were floated would influence the formation of starch in the leaf.

METHODS.—Using oxalic acid, the writers prepared two series of non-buffered solutions having pH values of 2.7, 3.1, 3.3, 3.6, 3.8, 4.0, 4.2, 4.4, 4.7, 4.9, 5.2 and 6.1. Enough glucose was added to the solutions of one series to give a molecular concentration of 0.45. Both variegated and non-green leaves were cut into strips and floated on the solutions, some with the upper surface and others with the lower surface exposed to the air, being sure that the cut margins were in contact with the solution. The leaf portions in the series containing no sugar were exposed to the light from a 200 watt, concentrated filament bulb at a distance of two feet, for fifteen hours before being tested for starch by the usual iodine method. The second series, containing sugar, was placed in diffuse light, other conditions remaining approximately the same as those of the first series.

The pH values of the solutions were re-determined at the end of fifteen hours.

RESULTS.—Table I shows the results obtained from the above experiment. The numbers used indicate the relative amounts of starch present in the tissues at the various H-ion concentrations. The number "1" indicates a trace of starch; "6" indicates an abundance of starch.

It may be noted from the table that starch was synthesized throughout the entire range in acidity in both the green and non-green tissues of the leaf portions floated on glucose in diffuse light. In the non-green portions, the starch was most abundant near the veins and cut edges. The maximum starch formation in the non-green portions occurred at pH 4.4, while the maximum amount in the green portions occurred in the solutions having a pH of 3.8—4.0.

TABLE I.

Table indicating the relative amounts of starch found in variegated leaf portions of *Pelargonium* floated on oxalic acid solutions of different pH values; one series in diffuse light containing glucose at a 0.45 molecular concentration, the other in continuous bright light, but containing no glucose. Further explanations may be found in the text.

pH		2.7	3.1	3.3	3.6	3.8	4.0	4.2	4.4	4.7	4.9	5.2	6.1
Series with Glucose.....	Non-green....	1	1	2	3	3	4	4	5	4	4	3	3
	Green.....	4	4	4	5	6	6	5	5	4	4	3	3
Series without Glucose.....	Non-green....	...	...	...	...	...	...	...	...	...	...	...	...
	Green.....	5	5	6	6	6	6	6	6	6	6	5	4

In the leaf portions of the series exposed to continuous light but having no additional glucose in the solutions, it was found that the green portions of the leaves contained abundant starch throughout almost the entire range, although starch was completely absent in the non-green portions.

Examination with the microscope revealed that while the starch grains exhibited the ordinary violet color associated with the iodine reaction, the vacuoles of the mesophyll cells of the leaves in the continuous light on the sugar-free solutions, contained a red staining substance, probably a dextrin, throughout the entire range of the experiment. The color was most intense at the ends of the series being less intense from pH 3.8 to 4.4. Neither starch nor dextrin could be detected in the non-green cells of the same leaves.

In the series floated on the sugar solutions in diffuse light, dextrin was detected in the green cells of the leaves from pH 2.7 to 3.6, and again from 4.9 to 6.0, practically no red color being detected from pH 3.8 to 4.7. In the non-green leaf portions, only a slight red color could be detected at the lower pH values, the color becoming more evident in the higher values, within the same range as that for the green portions.

At the end of the experiment it was found that little change had taken place in the pH values in either series up to 3.8, but beyond this point there was a gradual increase in the pH value of all solutions, none of them being higher than 6.2.

DISCUSSION.—The writers are aware that they can not state the effectiveness of oxalic acid in regulating the H-ion concentration of the cell contents of tissues floated on a solution of this acid; yet it is evident that in those leaves floated on sugar solutions, the maximum starch synthesis occurred in those having a pH value near the averages for the two portions of the leaf under greenhouse conditions, that is, pH 4.4 for the non-green tissue and pH 4.0 for the green.

It is found that some form of dextrin is an intermediate stage between the monosaccharides and starch in *Pelargonium*, although between pH 3.6 and 4.9 where the dextrin was found to be low or absent, this transition stage may be passed through more quickly than at lower or higher pH values.

It is possible that the acid conditions of the various solutions might so change the permeability of the cell membranes that at the different pH values a greater or less amount of soluble carbohydrate, if available, might diffuse into the tissues and be used in starch synthesis. This possibility, however, is thought to be small in this particular experiment, for although the green portions of the leaves in the series in continuous light were gorged with starch and dextrin, at no pH value had sufficient soluble carbohydrates diffused from a green cell into an adjacent non-green cell to bring about starch synthesis.

It is suggested that the carbohydrases in the green cells are sufficiently active to almost immediately change any photosynthetic product into an insoluble form, particularly within the pH range of the normal green tissue.

Whatever the effectiveness of the oxalic acid may be in regulating the acidity of the cell contents, it is quite evident that the two tissues synthesize starch most abundantly in

a range of pH values, the point of maximum activity for the non-green being somewhat higher than that in the green.

TEMPERATURE EFFECTS.

Since the greatest amount of starch in the non-green parts was found in leaves treated in the 0.5 molecular solutions, this concentration was selected for a study of the effect of temperature on starch synthesis.

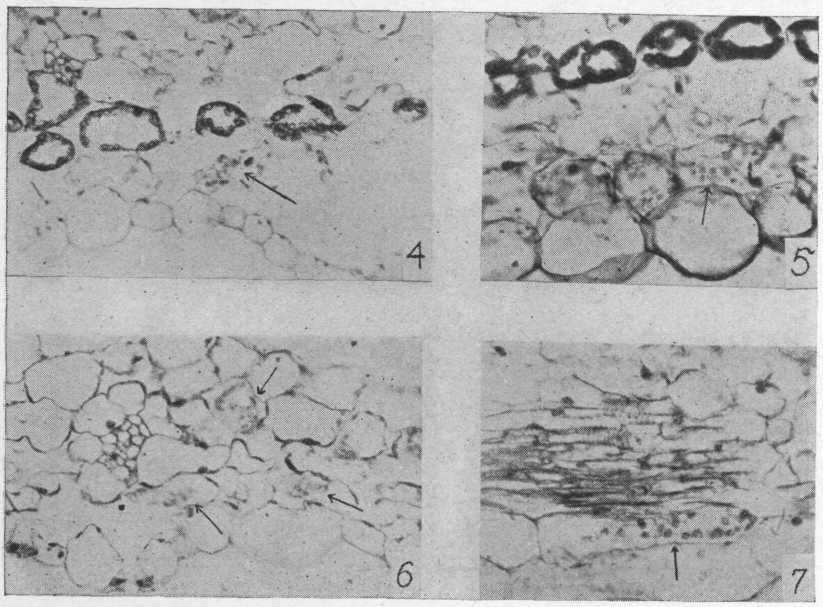
METHODS.—A series of 0.5 molecular glucose solutions, containing variegated leaves, was distributed in constant temperature chambers at 4°, 8°, 12°, 15°, 20°, 25°, 30°, 35°, 38°, 40°, 48°, and 50° C. At 38°, and above, large beakers were placed over the leaves to reduce transpiration. Starch tests were made at the end of fifteen hours. Microscopic examinations were made to detect any small quantities of starch present.

RESULTS.—A few of the leaves from the 4° C., chamber revealed a trace of starch in the cells of the albino mesophyll. Most of the leaves from the 8° C. chamber had a trace of starch, and all of the leaves from the 12° C., chamber showed a small quantity, yet a decided increase over the preceding. A very noticeable, steady increase of starch with increase of temperature appeared until a maximum was reached at 25° to 38° C. At these temperatures, the non-green portions were gorged with starch. Above 38° C., the amount of starch synthesized decreased until only a trace was found in a small number of leaves at 50° C.

DISCUSSION.—It has been recognized since the work of Böhm (1883) that temperature has an appreciable effect upon the amount of starch synthesized in leaf parts floated on sugar solutions. Winkler (1898) reported starch formation in treated leaf tissues of various species over a range of temperatures from 1° to 45° C., stating that the optimum range for most plants lay between 10° and 20° C. In the temperatures above 20° C., he found no further increase in starch.

Using a 0.5 molecular solution of glucose, the writers observed that the greatest yield of starch in the white parts of *Pelargonium* leaves occurred in those exposed to temperatures between 25° and 38° C. It should be remembered in comparing the present work with that of Winkler that he used the methods of Böhm and Meyer in his studies, that is, floating cut portions of leaves on sucrose solutions, while the writers

inserted the cut petioles in the solution. It might be that this difference in technique would produce slightly different results, but it is the opinion of the writers, based upon the preliminary studies where both methods were tried, that the difference is negligible.



FIGS. 4, 5, 6, 7. Cross-sections of the leaves of the variegated *Pelargonium*.

4, 5. Sections through the light-green areas showing the single celled layer of chlorenchyma, the large chloroplasts in the green cells, and the small leucoplasts in the non-green cells.

6. Section of the albino portion showing the weakly developed leucoplasts.

7. Section of an albino leaf near a vascular bundle, after several hours in a glucose solution, showing the early development of starch in the leucoplasts near the phloem. Arrows mark cells containing particularly well defined leucoplasts.

PLASTIDS.

In the variegated leaves of *Pelargonium*, there may be noted three distinct zones, green, light-green, and non-green. The writers made certain cytological studies to ascertain the distributional and structural differences of the plastids in these regions.

MATERIALS AND METHODS.—The material for this part of the work was taken from plants of the same clon culture as

that used in the physiological studies. Both fresh and killed material was examined. The fresh material was cut by hand and mounted either in water or a weak iodine solution. In the special preparations the material was killed in several fixatives. It was found, however, that an alcohol, corrosive sublimate, acetic acid, formaldehyde mixture gave the best results.

The pieces of leaf to be examined were imbeded in paraffin and sectioned in the usual way at thicknesses varying from 5 to 15 microns, and stained in Haidenhain's haematoxylin, according to the modified technique of one of the writers (Camp, 1931).

Owing to the difficulty of cutting sufficiently thin sections from leaves containing the large starch grains which occur in the non-green portion after treatment, the best preparations were obtained from material which was fairly free from starch.

RESULTS.—In the dark-green portions of the leaves, the chloroplasts were generally distributed in the cells of the spongy and palisade layers of the mesophyll, occasional cell layers being free of them. The light-green areas of the leaf generally contained but one layer of cells with chloroplasts (Figs. 4, 5), while the mesophyll cells of the non-green portions were free of them.

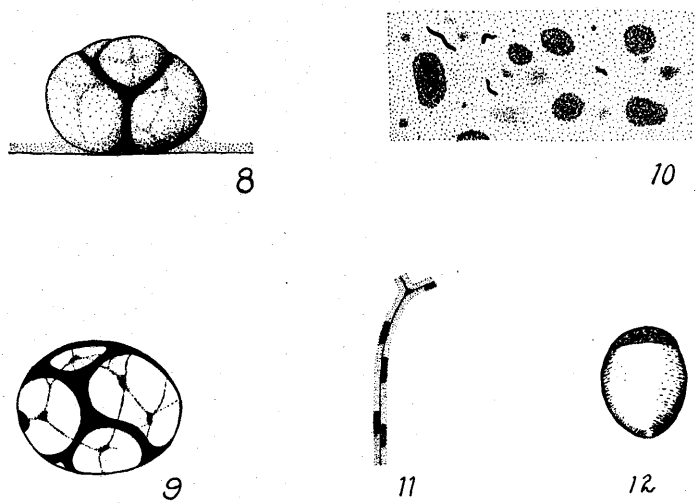
In the non-green portions of leaves which were not treated and which did not contain starch, small leucoplasts were found imbedded in the cytoplasm.

With the killing reagents and stain used, the plastids in the green portions of the leaves stood out very clearly, while those in the non-green portions were poorly defined, often appearing only as slightly denser masses of cytoplasm.

The two types of plastids differed greatly in their structure. The chloroplasts were somewhat uniform in size and shape, appearing as slightly lobed, spheroid structures (Fig. 8). Sections through the chloroplasts showed that they were vacuolate and contained 3 to 5 well defined chambers, supported and partially divided by deeply stained trabeculae which were much thicker than the plastid wall or membrane. Very minute branched strands also connected the trabeculae, but apparently were not a part of the primary structure of the plastid, although in a considerable number of plastids they seemed to have a definite arrangement in relation to the trabeculae (Fig. 9).

In contrast, the leucoplasts occurring in the starch-free, non-green portion showed no internal structure, appearing as irregularly sized, flattened granular masses or plates in the cytoplasm (Figs. 10, 11).

The leucoplasts were difficult to locate in fresh material, and even in the stained preparations were often poorly differentiated from the cytoplasm (Figs. 4, 5, 6, 10). The larger leucoplasts often showed several darker staining groups of granules within. Irregular, elongated and deeply stained bodies resembling chondriosomes also occurred in the cytoplasm of the non-green cells.



FIGS. 8, 9, 10, 11, 12. Plastids in the green mesophyll and leucoplasts and starch grains in the albino mesophyll of *Pelargonium*. (× 2300.)

8. External appearance of the chloroplast.
9. Internal structure of a starch-free chloroplast showing the trabeculae.
10. Surface view of starch-free leucoplasts showing the associated granules and chondriosome-like bodies.
11. Side view of starch-free leucoplasts.
12. Developed starch grain showing the cap-like leucoplast.

The two types of plastids were further contrasted in the manner in which they formed starch grains. In the chloroplasts starch was found in the chambers within the plastids, the number of grains corresponding to the number of chambers in the plastid.

In the leucoplasts, the starch is apparently synthesized on one side or face of the plastid, the starch grain, after considerable

growth, appearing roughly elliptic in outline, the plastid body forming a cap at the end of the grain (Fig. 12). If a plastid membrane is present around the mature starch grain, it is too thin to be detected.

While most of the starch grains in the non-green tissue were roughly elliptic in outline, some of them were irregularly shaped. Compound grains were fairly common, originating either in a single large, irregularly-shaped plastid with several centers of activity or by the crowding and coalescing of the grains from a group of closely associated small plastids.

Evidence gleaned from relative staining reactions, optical activity, and behavior at different temperatures, indicated that the starch formed by the chloroplasts and the leucoplasts is somewhat different.

No evidence of a gradual transition from the normal chloroplasts to the leucoplast was found, the two occurring in their extreme forms in adjacent cells. Nor could any evidence be obtained from the material examined to substantiate the claim of some writers that the leucoplasts of *Pelargonium* are degenerate chloroplasts. A study of etiolated and physiologically disorganized leaves did reveal a gradual break-down and degeneration of the chloroplast, but so far as the writers were able to ascertain they did not become transformed into leucoplasts.

DISCUSSION.—It is generally accepted at present that the plastids of many of the higher plants originate in chondriosome-like proplastids which occur in the meristematic cells. While the writers made no special study of this matter, it has been shown by several investigators (see Schürhoff 1924) that *Pelargonium* is no exception.

The problem of the origin of the two types of plastids is a difficult one to solve for there are undoubtedly a number of factors which influence their development. If both chloroplasts and leucoplasts arise from the same type of plastid primordia, there must be some factor or group of factors operating during the process of differentiation producing the two plastid types. Whatever the factors are that bring about the differentiation, they operate within a very narrow band of physiological activity, for immediately adjacent cells contain chloroplasts or leucoplasts in their extreme forms, and at no time could transition or intermediate plastid types be found.

It is also shown by sections of very young leaves that the differentiation takes place early.

Although it is well known that leucoplasts at certain times may form chlorophyll and function as chloroplasts, no evidence that this is true was found in *Pelargonium*, the variegated areas of the leaves remaining the same from the early stages until senility.

An examination of a single plant gives ample evidence that the differentiation is due to a set of factors which are more effective at one time than at another, for certain leaves will show but little variegation, only a thin margin of non-green tissue being in evidence, while other leaves may be almost entirely white.

The writers' observations indicate that light intensity affects the internal conditions influencing differentiation, for plants grown in bright sunlight tend to have but little non-green tissue, while those grown at the same temperature and humidity, but at a lower light intensity, have leaves with more non-green tissue. Individual leaves occasionally approach an almost complete albino condition.

It is also probable that the physiological gradients of the developing leaf are a factor, for the non-green portion is invariably at the margin of the leaf, and if only a small amount of chlorenchyma is present, it is near the base of one or more of the large veins of the leaf blade.

That the variegated habit is also hereditary has been shown by Baur (ref. in Schürhoff 1924), for crosses between green and variegated plants of *Pelargonium* gave variegated and non-variegated segregates in succeeding generations.

The plastids are morphologically differentiated, for the chloroplasts are irregular spheroids made up of 3 to 5 chambers, the starch, when formed, being deposited within the chambers. The leucoplasts, when starch-free, are discoid structures, often irregular in outline and sometimes optically but poorly differentiated from the granular cytoplasm. When formed, the starch is deposited by one face of the leucoplast, the active portion forming a cap over the end of the starch grain.

Haberlandt (1914) and Zirkle (1926) have shown that this type of starch-forming plastid is vacuolate, the starch grain arising internally and bursting through the plastid after growth. Although some evidence of this could be found in the leucoplasts of the material examined during the first stages of starch

formation (Fig. 7), it is to be remembered that later, only one face of the plastid is ordinarily active, the starch accumulating at the face of the greatest activity.

Reichert (1913) pointed out that starches differ greatly in their physical and chemical properties, and although no extended study of this point has been made by the writers, there were indications that the starches formed by the chloroplasts and leucoplasts of *Pelargonium* differ in their optical properties, iodine reaction, and probably in their gelatinization temperature.

CONCLUSIONS.

In the study of the synthesis of starch in the green and non-green portions of the leaves of the variegated pelargonium (*Pelargonium hortorum* var. *Mme. Salleroi*), the writers came to the following conclusions:

1. Starch synthesis normally does not occur in the non-green portion of the *Pelargonium* leaf, but will occur if the leaf is placed in a solution of glucose, the optimum point for starch formation being approximately a 0.5 molecular concentration.

2. Under normal conditions in the greenhouse, the green tissue contains approximately twice as much sugar as the non-green tissue.

3. Although the non-green tissue of the leaf consistently has a higher pH value than the green tissue, it is thought from comparative studies of *Pelargonium* and other leaves that this condition is not so much correlated with the type of tissue as with its position in the leaf.

4. Cut portions of the leaves, when floated on sugar free oxalic acid solutions of pH values ranging from 2.7 to 6.1 and exposed to fifteen hours of continuous light, synthesize starch in the green tissue; and although the carbohydrates were abundant in the green tissue, no evidence of starch synthesis could be found in the non-green portions. Since at none of the pH values could starch be found in the non-green portions, it is probable that in this type of experiment and under normal conditions in the leaf, the carbohydrases present in the green cells, by hastening the condensation of the photosynthetic products, keep the concentration of soluble carbohydrates in the green cell very low. The sugar in the adjacent non-green cells, in equilibrium with that in the green cells, is, therefore,

still below the threshold for starch synthesis for that type of tissue.

5. When cut portions of the leaves are floated on oxalic acid solutions containing glucose of pH values ranging from 2.7 to 6.1, and exposed to diffuse light, starch is synthesized in both the green and non-green portions; and since the starch is most abundant along the veins and cut edges, this indicates that each portion may independently synthesize starch when supplied with glucose.

6. The pH value of the normal tissue is not the limiting factor in the lack of starch synthesis in the albino portion under greenhouse conditions for the greatest amount of starch is synthesized by the non-green tissue when floated on a glucose solution with a pH value of 4.4, the value for this tissue under normal conditions.

7. When glucose is artificially supplied, starch is synthesized equally well in the green and non-green portions of the leaf at temperatures ranging from 4° to 50° C., indicating that temperature is not a factor in the lack of starch synthesis in the non-green portion of the leaves.

8. The chloroplasts of the green tissue and leucoplasts of the non-green tissue are differentiated in the young leaf while in the primordial condition, nor is evidence found of intermediate or transitional forms to indicate that the leucoplasts are merely degenerate chloroplasts.

9. Although the leucoplasts and the chloroplasts are morphologically different, they are both able to synthesize starch if supplied with glucose.

10. Although there are a number of factors which influence starch synthesis in the non-green portion of the *Pelargonium* leaf, the factors probably directly responsible for the absence of starch under greenhouse conditions are the low concentration of soluble carbohydrates in this tissue and the different points of sugar-starch equilibrium in the green and non-green cells.

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